Supporting Information for

Methylsorb: A simple method for quantifying DNA methylation using DNA-gold affinity interactions

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Table S1. Summary of primer sequences employed in this study.

SAMPLE AMPLIFICATION		
Short name	Description	Sequence
<i>En1</i> Fw-primer	Forward primer for asymmetric PCR of <i>EN1</i> region	5'-ATTCAGTCCACAACAAYGTTGGTTGAGTTTATAAGTAGGATAGT-3'
<i>En1</i> Rv-primer	Reverse primer for asymmetric PCR of <i>EN1</i> region	5'-ACRACCRCAACAACCAAACCT-3'
<i>MIR200B</i> Fw-primer	Forward primer for asymmetric PCR of <i>MIR200B</i> region	5'-TTAATAAGTTAGGTTTTAYGTYGGGATTATATT-3'
<i>MIR200B</i> Rv-primer	Reverse primer for asymmetric PCR	5'-AATCAAAAAACCCAAAACRCCCAAC-3'
SAMPLE QUANTIFICATION		
<i>Col2A1</i> Fw-primer	Forward primer for quantifying DNA using the <i>Col2A1</i> gene	5'-GTAATGTTAGGAGTATTTGTGGGTA-3'
<i>Col2A1</i> Rv-Primer	Reverse primer for quantifying DNA using the <i>Col2A1</i> gene	5'-CTACCCCAAAAAACCCAATCCTA-3'
SAMPLE SEQUENCING		
<i>EN1</i> SeqFw-primer	Forward primer used for sequencing of <i>EN1</i> region	5'-GAGGTTGTTGTGAGTTAGTTTTGATT-3'
<i>EN1</i> SeqRv-primer	Reverse primer used for sequencing of <i>EN1</i> region	5'-ACRACCRCAACAACCAAACCT-3'
<i>MIR200B</i> Seq Fw-primer	Forward primer used for sequencing of <i>MIR200B</i> region	5'-TTATTTTATTTTAGTTTGTAGGTGGGA-3'
<i>MIR200B</i> Seq Rv-primer	Reverse primer used for sequencing of <i>MIR200B</i> region	5'-AACCAAACTCAACTTAAAAAATCCAAAA-3'

Table S2. *In silico* analysis of poly AC sequence motifs within DNA Methylation biomarkers from cancer related genes. Sequences from methylated and unmethylated epigenotypes were generated using BiQ software. DNA sequence motifs of minimum 4 identical bases are underlined; sequence motifs corresponding to poly A/C mixed bases of minimum 4 nucleotides in length are highlighted in grey colour; the A/G differences between the methylated (M) and unmethylated (UM) strands are highlighted in red font.

Biomarker		DNA Antisense strand after bisulfite conversion and asymmetric PCR	Remarks
RASSF1 ¹ (Breast Cancer)	UM	5'-ACACCATTACCTA <u>ACCCCTCCC</u> ACCTCAAAATCACTAAC AAAAAAAAAAACAAACAACCA-3'	Long poly-AC motif
	M	5'-GCGCCATTGCCTAGCCCTCCC <u>G</u> CCTCAAAATC <u>G</u> CTAG CAAAAAAAAAAGCAAGCAGCCA-3'	Short poly-AC motif
DAPK ¹ (Breast Cancer)	UM	5'-AACAAACACAAAACCACCCAACAAAAAAAAAAAAAAAAAA CAAAAAAACCCACCAACATACAACCCAACCAACCCA-3'	Long poly-AC motif
	M	5'-AACAAACGCAAAGCCACCCA <u>G</u> CAAAAAAAAAAAAAAAAAAA ACAAAAAAGCCCGCCAGCATGCAAGCCAGCCAGCCCA-3'	Short poly-AC motif
DKK3 ² (Breast Cancer)	UM	5'-CACCACACCACCCACCAACACCCACACCCCTCACAC-3'	Long poly-AC motif
	M	5'-CGCCGCGGCCACCCGCCAGCGCCACGCCCTCGCGC-3'	Short poly-AC motif
MGMT ¹ (Colorectal Cancer)	UM	5'-ACAAAAACACACAAAACCAAAACAAAAACACACA-3'	Long poly-AC motif
	M	5'-GCAAAAGCGCGCAAAAGCCAAGCAAAAGCGCGCA-3'	Short poly-AC motif
MIR200B ^{3,4} (Bladder and Breast Cancer)	UM	5'-CTACAAAACACACTCCCCATAAATACACCCCTCAACA CACCTAACCAACCTACCCACCTACCTAACCAAA-3'	Long poly-AC motif
	M	5'-CTACGAAACACACTCCCCGTAAATACGCCCTCGAC GCACCTAACCGCCCTACCCGCCTACCTAACCGAA-3'	Short poly-AC motif
SHOX2 ⁵ (Lung Cancer)	UM	5'-AACAAACAACAAAACAAAAAAAAACAACAACAAAAC AACCAACAACCTAACCAACCA-3'	Long poly-AC motif
	M	5'-AGCAGCAGCAAAAGCAAAAAAGCAGCAGCAAAAGC AGCCAGCAAGCCTAACAGCCCA-3'	Short poly-AC motif

Figure S1. Detection of DNA methylation in synthetic samples within the selected EN1 region at pH 3. SPR sensorgrams showing the spectral shift generated during the adsorption of methylated (red) and unmethylated (blue) synthetic EN1 region onto the gold chip at pH=3.

Figure S2. Sanger sequencing of sense strand containing the eight CpG sites region within MIR200B gene in bisulfite treated MDA-MB-231 DNA.

PCR bias estimation. PCR-bias is one of the major problems in most existing PCR-based DNA methylation detection approaches. It arises as a consequence of Bisulfite treatment which changes sequence composition of methylated and unmethylated templates. Since amplification by PCR has strong sequence-dependence efficiency, bisulfite treatment on DNA can cause that one population of sequences (usually the unmethylated sequence) may amplify preferentially⁶ resulting in an inaccurate estimation of DNA methylation in heterogeneous samples containing an excess of unmethylated sequences. To evaluate the effect of PCR bias on the Methylsorb approach, we theoretically calculated the adsorption level for a given methylation percentage and compared this with the experimental value. Initially, the experimental value for a given methylation percentage was normalized by subtracting the offset value generated by the 100% methylated sample (*i.e.*, $S_X - S_M$, where S_X is the adsorption provided by the X% methylation and S_M is the value provided by the 100% methylated sample). The theoretical value for a given percentage (*i.e.*, X% methylation) was then estimated using the following equation-

$$NPS = \frac{(S_{UM} - S_M) * (100 - X)\%}{100\%} \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (1)$$

where NPS is the normalized predicted SPR (NPS) shift for the sample containing X% DNA methylation, S_{UM} and S_M are the adsorption values resulted from 100% unmethylated and 100% methylated samples. We compared our normalized experimental values of each methylation percentage resulted from the *EN1* region on MCF7 and WGA DNA with those of the theoretical NPS values. It was found that normalized experimental values were slightly deviated from the theoretical NPS values (supplementary Figure 3). Clearly, the difference was within the experimental errors, and probably statistically insignificant. The chances of the PCR bias in our approach were significantly reduced by (i) controlling the gene copy

numbers of templates prior to the asymmetric PCR amplification and (ii) design of primers and optimization of the annealing temperature.

Figure S3. Estimation of the PCR bias during detection of methylation in heterogenous samples. The normalized spectral shift of predicted (black) against experimental (red) values were plotted for the different methylation percentages. A slightly higher divergence between both was noted indicating the presence of a very minor PCR bias towards the unmethylated epigenotype (higher adsorption value in the experimental SPR shift).

Supplementary References

- S1. Duffy, M.J. et al. *Eur. J. Cancer* **2009**, 45, 335-346.
- S2. Kloten, V. et al. *Breast Cancer Res.* **2013**, 15, R4.
- S3. Köhler, C. et al. *Biochem. Biophys. Res. Commun.* **2013**, 438, 48-53.
- S4. Lim, Y. et al. *J Cell Sci.* **2013**, 126, 2256-2266.
- S5. Schmidt, B. et al. *BMC Cancer* **2010**, 10, 600.
- S6. Warnecke, P.M. et al. *Nucleic Acids Res.* **1997**, 25, 4422-4426.